
Diarrhetic Shellfish Toxin and Lipophilic Toxin Profiles in Japanese Bivalves and an Effective Monitoring System by Using a Rapid Assay Kit

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Development of rapid screening methods for lipophilic toxins and paralytic shellfish toxins in Japan (2003-2006)

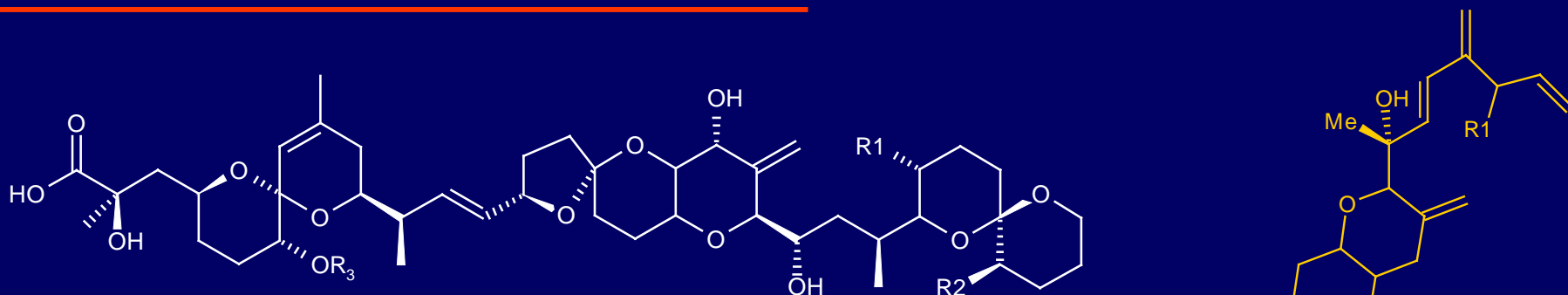
- Period: 2003-2006
- Funded by the Japanese government
- Budget: 450,000 US \$ per year



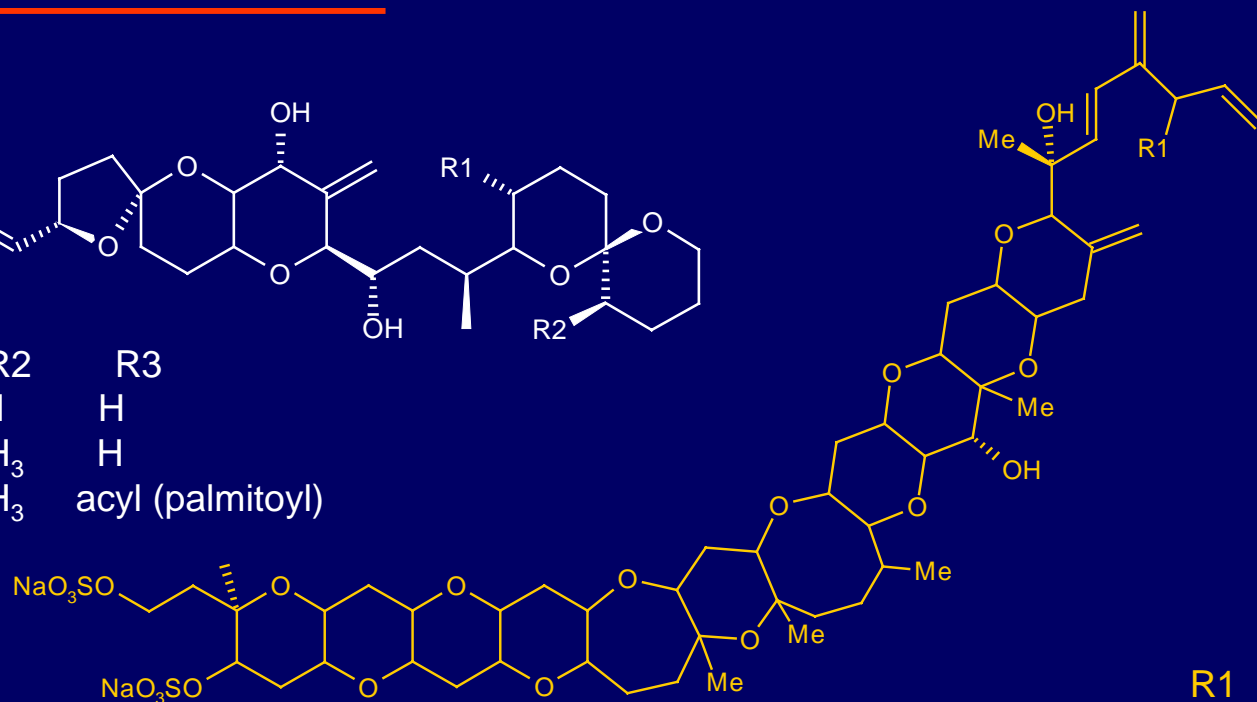
Development of rapid screening methods for lipophilic toxins and paralytic shellfish toxins in Japan (2003-2006)

- OA PP2A assay kit (Japan Food Research Lab.)
- YTX ELISA kit (Japan Food Research Lab.)
- PTX ELISA kit (Japan Food Research Lab.)
- PSP ELISA kit (Osaka Pref. Inst. for Public Health)
- Instrumental methods (HPLC, LC-MS) for confirmatory tests of screening methods (Tohoku Univ., Tohoku Natl. Fish. Res. Inst.)
- Background data
 - Toxin profiles of toxic plankton and bivalves
 - Screening assay kit vs HPLC(LC-MS), etc
 - (Tohoku Univ., Tohoku Natl. Fish. Res. Inst.)

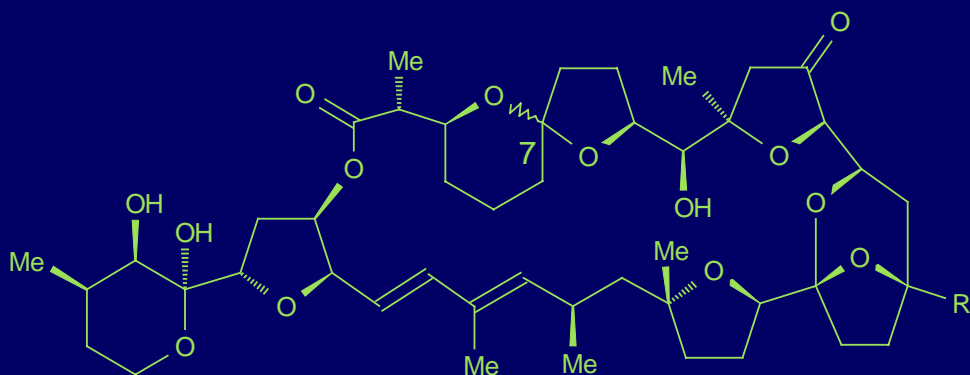
Lipophilic toxins found in Japanese bivalves



	R1	R2	R3
Okadaic acid	CH ₃	H	H
Dinophysistoxin-1	CH ₃	CH ₃	H
Dinophysistoxin-3	CH ₃	CH ₃	acyl (palmitoyl)

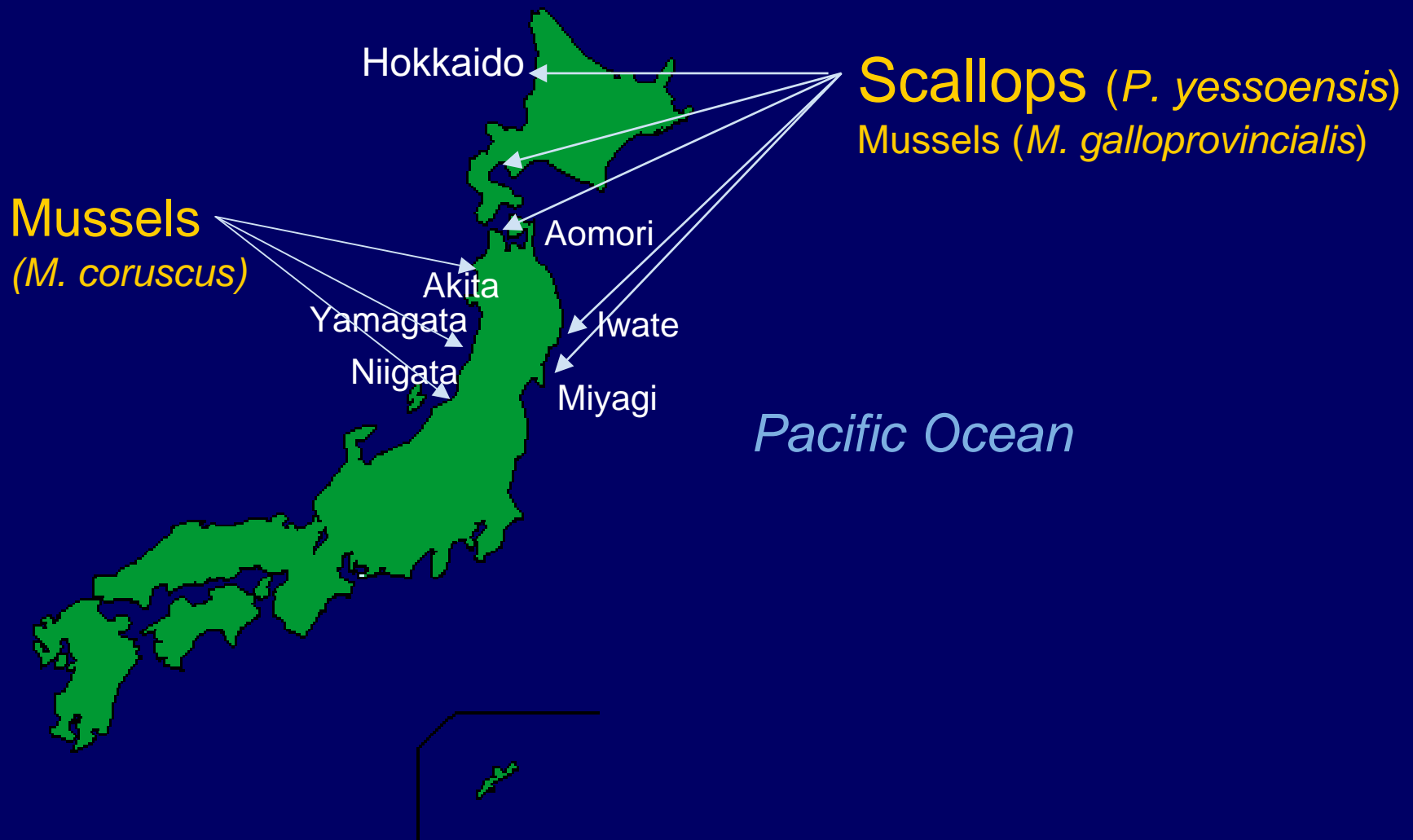


Yessotoxin	R1
45-Hydroxy-yessotoxin	H
	OH



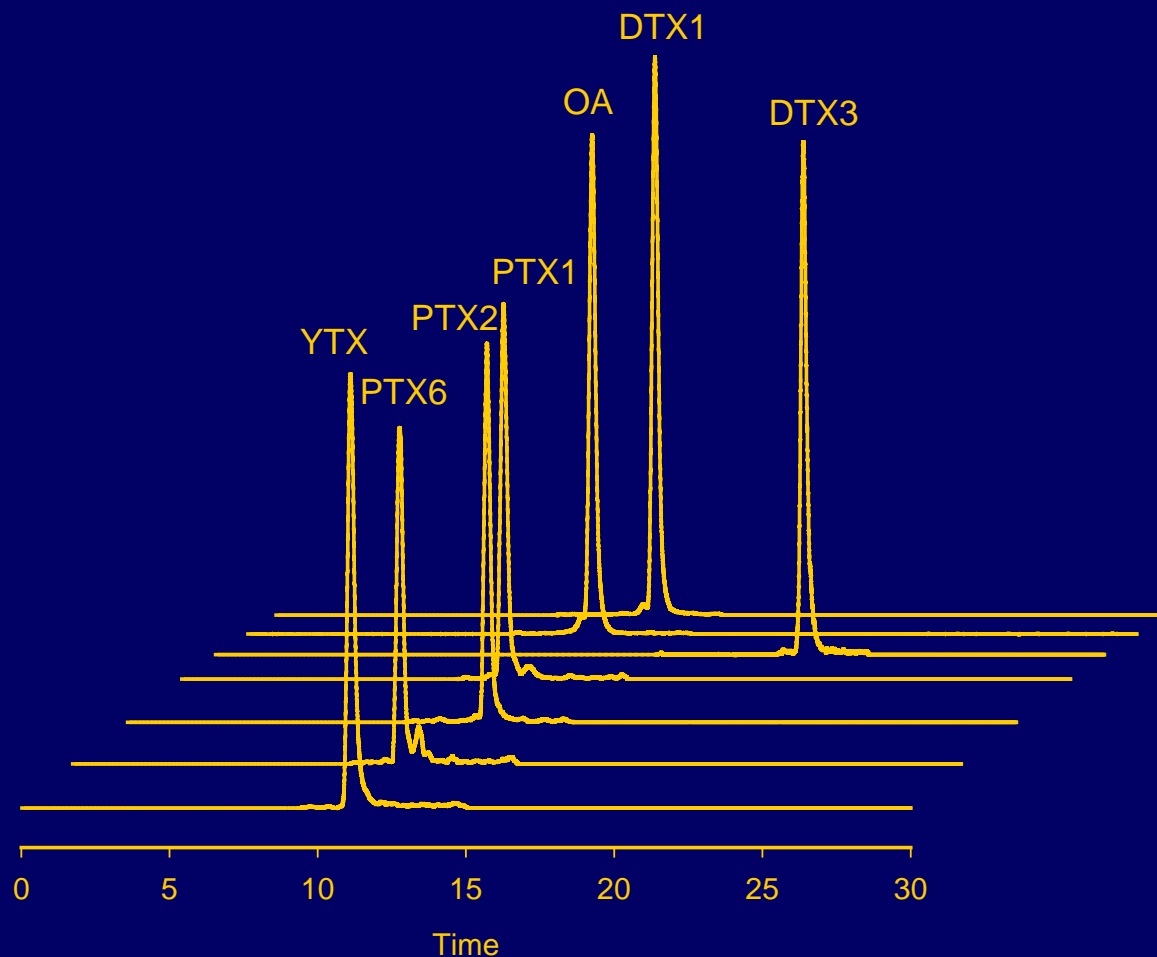
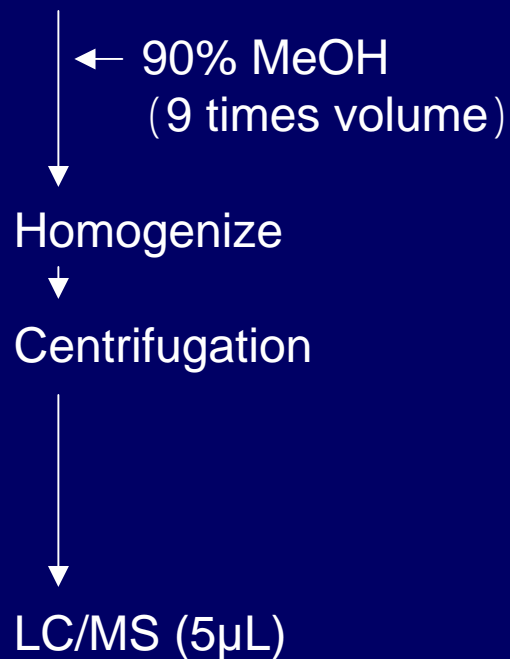
	R1	C7
Pectenotoxin-1	CH ₂ OH	R
Pectenotoxin-2	CH ₃	R
Pectenotoxin-3	CHO	R
Pectenotoxin-6	COOH	R

Production areas where harvesting was ceased due to contamination of shellfish with lipophilic toxins in 2003 and 2004

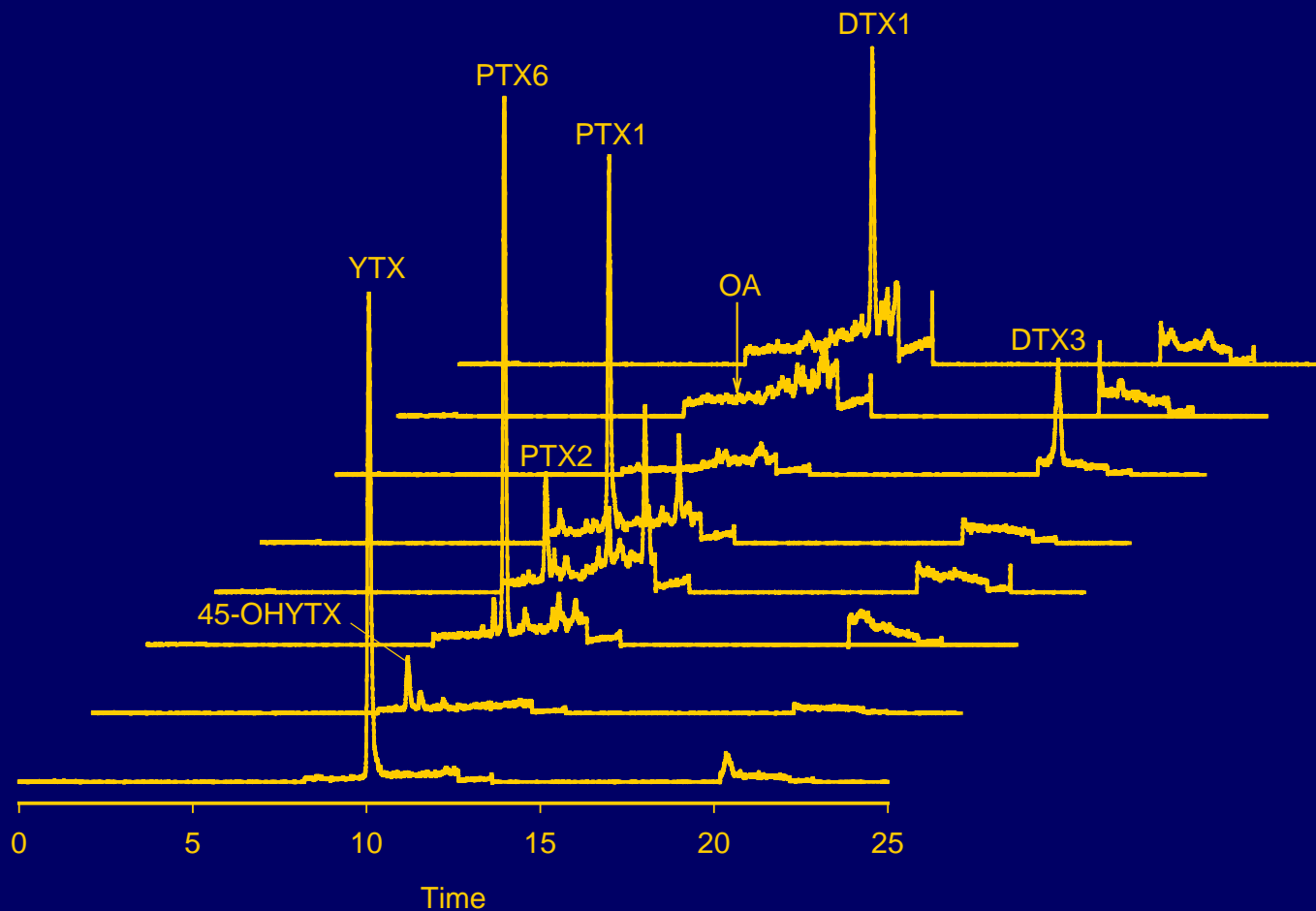


LC-MS analysis of lipophilic toxins in Japan

Hepatopancreas



LC-MS analysis of lipophilic toxins in HP of scallops



Recovery (%) of toxins spiked into 90 % MeOH extracts of scallop hepatopancreas^{*1}

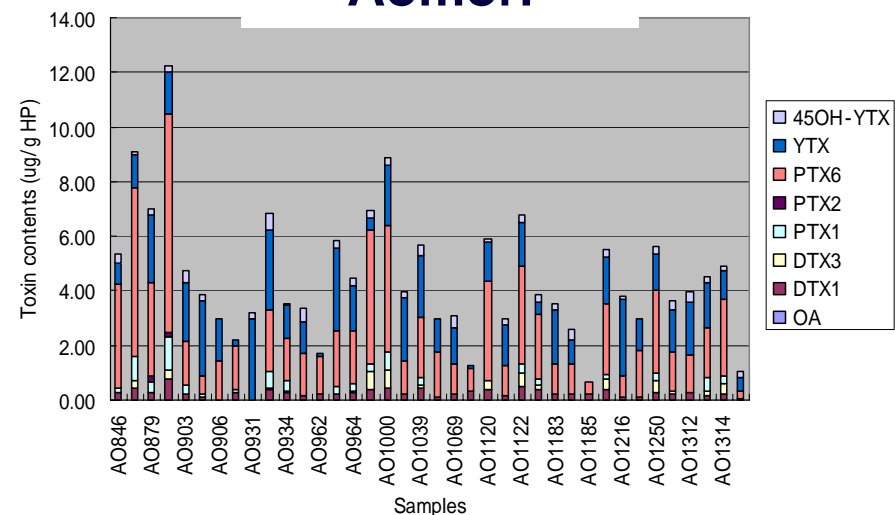
PTX1	92 ± 12 ^{*2}
YTX	97 ± 4
PTX6	106 ± 7
OA	90 ± 8
PTX2	93 ± 7
DTX1	94 ± 7
DTX3	109 ± 11

*1 Each toxin was spiked to the extract at a concentration of 0.5 µg/g.

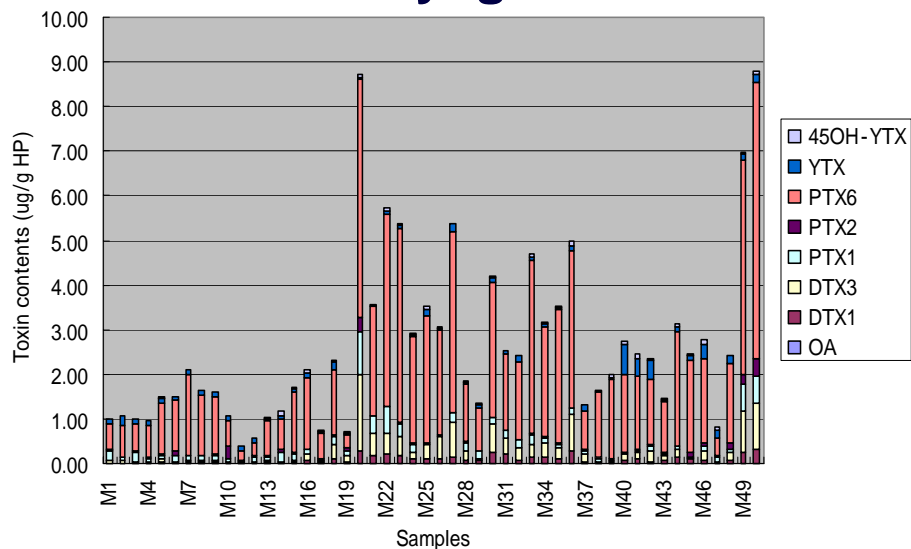
*2 Average ± standard deviation (n=5)

Toxin profiles of scallops (*P. yessoensis*) collected in 2003

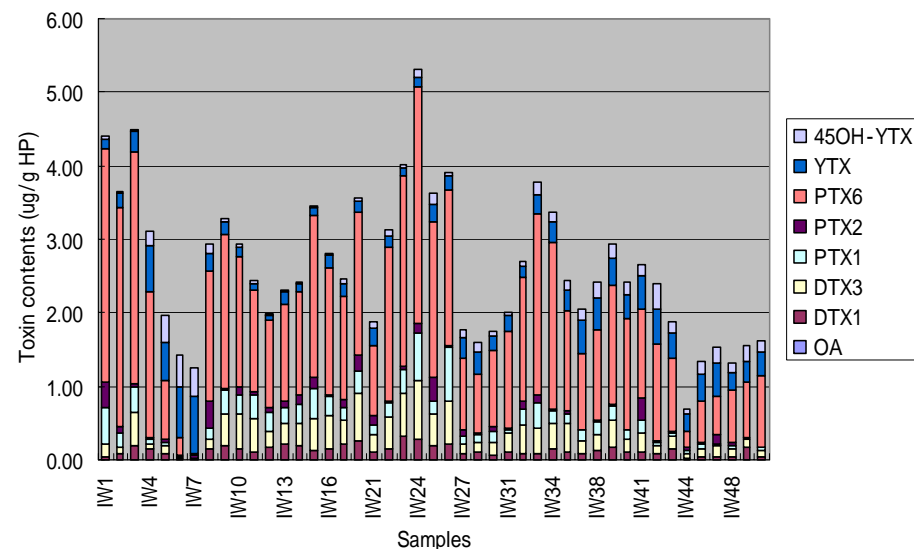
Aomori



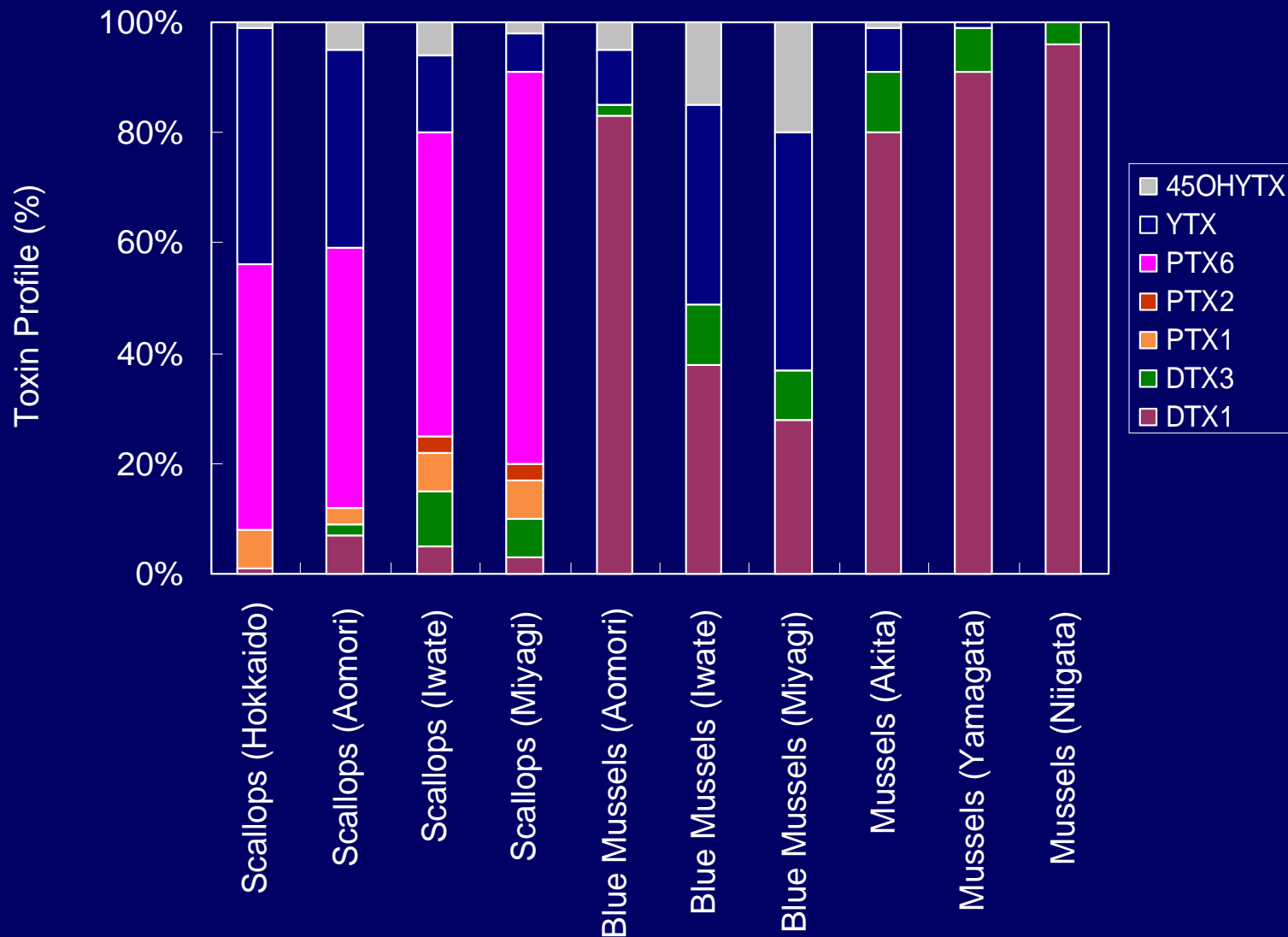
Miyagi



Iwate



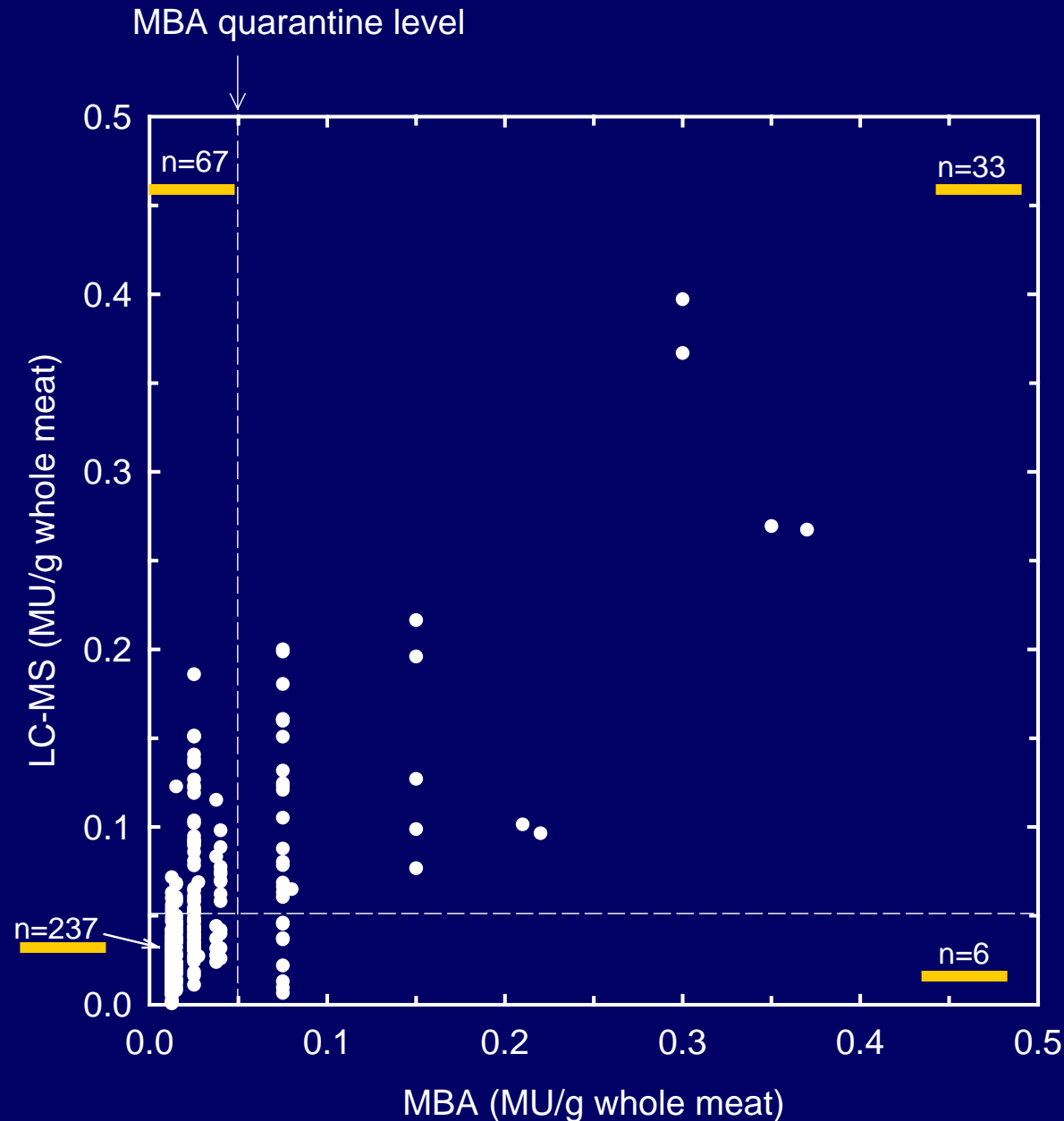
Dominant toxins in bivalves in Japan



Dominant toxins in bivalves in Japan

	<i>Dinophysis</i> spp.	<i>Protoceratium reticulatum</i>
	DTX1 PTX2	YTX
Scallops <i>P.yessoensis</i>	PTX6	YTX 45OHYTX
Mussels <i>M.galloprovincialis</i>	DTX1	YTX 45OHYTX
Mussels <i>M.coruscus</i>	DTX1	YTX 45OHYTX

Comparison of total mouse toxicities obtained by LC-MS and MBA

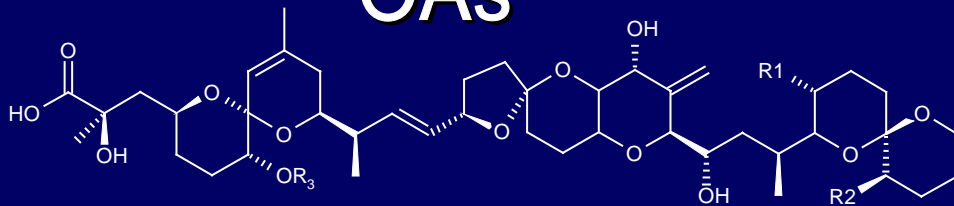


Average toxin profiles obtained from the 67 samples that gave the discrepancies between the toxicities (MU/g whole meat) quantified by MBA (<0.05) and LC-MS (>0.05)



Principle of PP2A inhibition assay

OAs



	R1	R2	R3
Okadaic acid	CH ₃	H	H
Dinophysistoxin-1	CH ₃	CH ₃	H
Dinophysistoxin-3	CH ₃	CH ₃	acyl (palmitoyl)

PP2A

MW(Active subunit)
: 35 kDa

Binding

Inactivation of PP2A

pNPP(Colorless)

Hydrolysis

pNP(Yellow)

- PO₄

pNPP(Colorless)

Hydrolysis

No color

pNPP : p-Nitrophenyl Phosphate
pNP : p-Nitrophenol

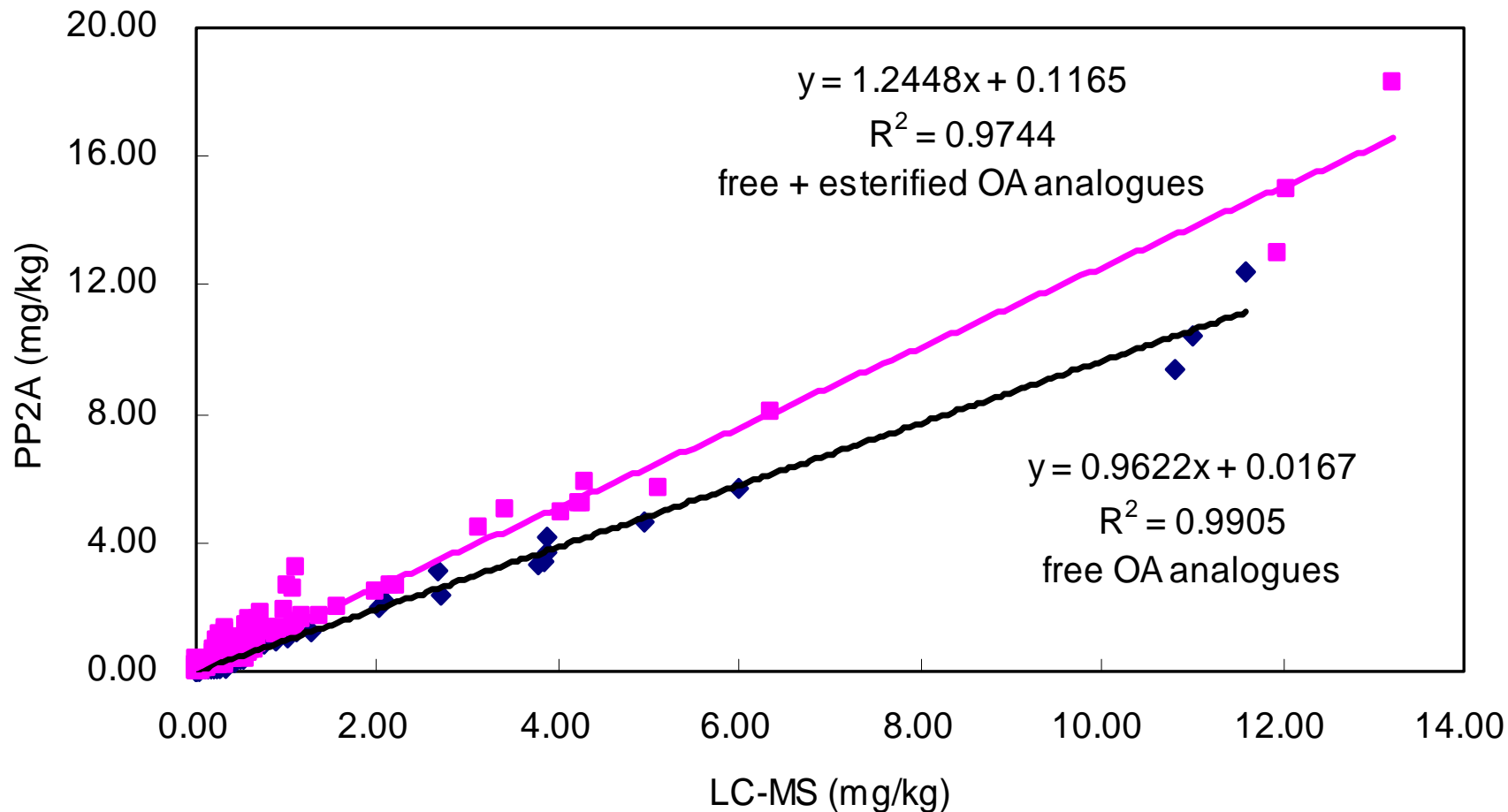
PP2A was purified
from *Neptunea* spp.



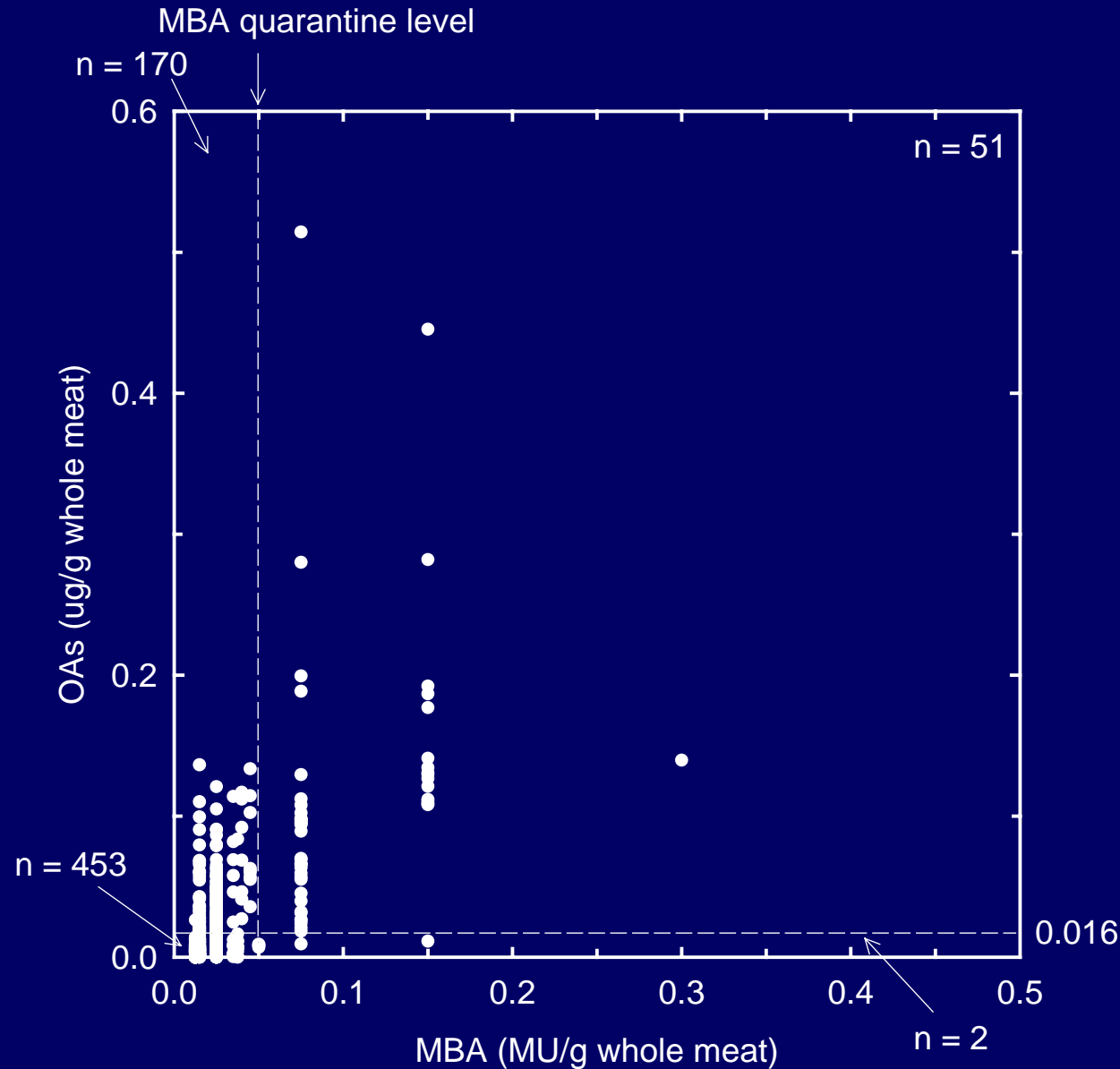
PP2A inhibition assay kit



LC-MS vs protein phosphatase 2A inhibition assay^{*1} for okadaic acid analogues



Comparison of mouse toxicities obtained by MBA and total OA contents quantified by LC-MS for scallops (*P. yessoensis*)



Percentage (%) of the numbers of MBA reduced when the the screening level (0.016 mg/kg whole tissues) is applied to the initial screening test in MBA

	Sample Numbers	MBA > 0.05 MU/g	OAs > 0.16 mg/kg	OAs > 0.016 mg/kg	%
Scallops	676	53	9	221	67
Blue Mussels	136	20	4	50	63
Mussels	36	13	12	32	11

Conclusions

- PTX6 and DTX1 are the most dominant toxins in scallops and mussels respectively, whereas YTX is a dominant toxin in both scallops and mussels.
- Comparison of the quantitative results obtained for more than 350 bivalve samples between LC-MS and mouse bioassay (MBA) showed that many samples assayed as being below the quarantine level (0.05 MU/g whole tissues) by MBA were quantified as exceeding the quarantine level by LC-MS
- Almost all of the samples quantified as exceeding the quarantine level (0.05 MU/g whole tissues) by MBA contained a level exceeding 0.016 mg/kg as the amounts of OA, DTX1 and DTX3 in whole tissues of bivalves
- More than 60 % of the numbers of MBA were reduced when this level was applied to the initial screening test in MBA

Acknowledgements

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